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MAY 29 2008

REMARKS / ARGUMENTS

The number of the U.S. provisional application has been corrected on page 1 of the specification to correct an obvious typographical error.

A revised declaration is being obtained which correctly identifies the filing date of the Canadian application. The applicant acknowledges with appreciation the correction suggested by the examiner.

A revised information disclosure statement is being prepared and will be submitted under separate cover.

With respect to the objections to the informalities in the specification, corrections have been made on pages 52 of the disclosure to correctly identify reference to numbers 4 and 5 rather than numbers 2 and 3.

The declaration is being corrected to correct the filing date of the Canadian application.

Claim 1 has been amended and the letter (c) has been corrected to refer to (a).

The informality previously contained in claim 12 has been corrected.

Claims 11 and 19 have been deleted.

With respect to the objection by the examiner relating to claims 1 to 7, the applicant intends for the amplified sequence to encompass and include the polymorphic position. Claim 1 has been amended to include this statement.

With respect to the objection to claims 1 to 7 over recitation of the phrase "the amplified *ob* gene polymorphism sequences", the subject matter of claim 4 has been inserted into claim 1 to provide a proper antecedent. Claim 4 has been deleted. Accordingly, all the claims are now dependent upon a proper antecedent.

With respect to the phrase "based on the presence of a particular *ob* gene polymorphism", the applicant intends for the identification to be based on the finding that the nucleotide content at a specific polymorphic position in the *ob* gene. The applicant intends to do such for both alleles present in every animal, that is, a CC genotype versus CT genotype versus TT genotype. Claim 1 has been amended.

The claims have been amended and now are restricted to a bovine species.

Reference to the greater feed conversion efficiency has been deleted from claim 3.

With respect to objection to claims 8 to 11, 20, 21 and 22, the applicant traverses the objection by the examiner.

If animals are screened for their genetic predisposition of milk production due to the *ob* gene, then the method of knowing to select for a T allele will increase the milk production. The milk production is definitely increased by this method as any group will by default be split into subgroups, and each subgroup will have an altered milk production status. Groups with a higher proportion of T alleles will have improved milk production. The selected group will have improved milk production based on the methods described, that is, increasing the T allele frequency.

The examiner has objected to claims 12, 13, 20, 21 and 22. The objection by the examiner is traversed.

There is a distinct way that an animal transfers his or her genetic material on to their offspring. If an animal is TT at said polymorphism, that indicates that both of the chromosomes in the particular chromosome pairing are harbouring a T allele; which means they can only transfer a T allele to their offspring in any breeding method/scheme. If an animal is CC at said polymorphism, that indicates that both of the chromosomes in the particular chromosome pairing are harbouring a C allele; which means they can only transfer a C allele to their offspring in any breeding method/scheme. If an animal is CT at said polymorphism, that indicates that one chromosome is harbouring a T allele and one chromosome is harbouring a C allele in their particular chromosome pairing; which means they can transfer either a C or T allele to their offspring in any breeding method/scheme – with a 50% likelihood of either a C or T being transferred at any given time.

The examiner has objected to claims 14, 15, 20, 21 and 22. The step of breeding has been inserted.

The examiner has objected to claims 16 to 22. The objection by the examiner is traversed. Milk production in this case will be increased because animals will be screened for the polymorphism and animals harbouring at least one T allele will be included in a new sub group – which will have an increased proportion of T alleles; which has been demonstrated to increase milk production.

The examiner has objected to claims 2, 3, 10, 11, 13, 18 and 19. It is clear that the applicant intends to claim an animal with a particular nucleotide content at a specific polymorphic position in a specific gene, the *ob* gene.

The examiner has rejected claims 1 to 22 in view of 35 U.S.C. 112, first paragraphs. Reconsideration and withdrawal of the objection by the examiner is respectfully requested.

The claims of the present application have now been restricted to bovines and bovine dairy cattle.

SEQ ID NO:4&5 are primers for a very specific region of the bovine *ob* gene which includes the polymorphic site which has a C to T transition. SEQ ID NO:6&7 are the fluorescently labelled hybridization probes which bind to an area within the region covered by SEQ ID NO:4&5 and will be the complement to the sequence where the SNP resides. That is, SEQ ID NO:6&7 are designed to have significantly similar homology to the wild type (C allele), and any chromosome which harbours the T (mutant) allele will have a reduced proportion of

homology to the wild type sequence. Because of this SEQ ID NO's: 4-7 are very specific for detecting an *ob* gene polymorphism. Since the claims have now been restricted to bovine animals and bovine dairy cattle, the objection by the examiner is traversed.

SEQ ID NO:1 & SEQ ID NO:2 are specific sequence which was originally generated from sequencing purified gel electrophoresis product of amplified bovine DNA – which was later entered into GenBank. Therefore it is a very specific limited DNA sequence generated from actual bovine amplified DNA, and is not able to change over time even though GenBank records admittedly can.

SEQ ID NO's :4-7 are small oligonucleotides which are part of SEQ ID NO:1 & SEQ ID NO:2, which are primers and hybridization probes from *ob* gene sequence. Since the primers and hybridization probes (SEQ ID NO's :4-7) are designed to be complementary to SEQ ID NO:1 & SEQ ID NO:2 it is very limited in scope of where the actual primers and hybridization probes will anneal and have utility. Therefore, they will be limited in scope of usefulness even in the *ob* gene.

Any bovine dairy animal may be used to determine whether the animal harbours a C or T on each chromosome by using SEQ ID NO's:4-7. That is SEQ ID NO:4 & SEQ ID NO:5 are the primers which amplify the specific region of SEQ ID NO:1 and/or SE ID NO:2. As shown in the tables it is necessary to look at the associations between milk production and the T or C allele. Because genomics are indeed predictive of biological systems behaviour, once an association has been established between an allele (i.e. T allele) and milk production it allows one to *a priori* identify the genotype (using SEQ ID NO's:4-7 & FRET melting curve analysis) and be indicative of the phenotype, i.e. increased milk production.

Since the claims are now restricted to bovine dairy animals, there is no contemplation of genotyping for the *ob* gene in any organism and thus the objection by the examiner is now moot.

The examiner has rejected claims 1 to 22 in view of 35 U.S.C. 112, first paragraph. The objection by the examiner is now moot since the claims have been restricted to bovine and bovine dairy cattle.

Also, the feed conversion efficiency has been deleted.

SEQ ID NO's:4,5,6, and 7 are the primers (SEQ ID NO:4&5) and hybridization probes (SEQ ID NO:6&7) for the Arg25Cys of exon 2 in the bovine *ob* gene. SEQ ID NO's:4,5,6, and 7 are designed to carry out Frequency Resonance Energy Transfer (FRET) analysis with temperature melting curve genotyping.

The instant specification does only contemplate the functional polymorphism as listed in position 189 of SEQ ID NO:1 & 2. This is because this polymorphism has been shown to have an association with these phenotypes (increased milk production and increased feed conversion efficiency); and it is also a particular polymorphism which is in an exon as well it changes the amino acid produced; therefore it is functional.

The applicant suggests to the examiner that it is not correct to suggest that the level of unpredictability with regard to associating the presence of a nucleic acid sequence or any

particular phenotype is high. It is suggested that the level of predictability that is significant at 95% is what is accepted by the scientific community.

While SEQ ID NO's:4-7 are not the sequence with the highest degree of homology with respect to SEQ ID NO's:1&2, they are nonetheless homologous to this region, and indeed span the region which encompasses position 189 of SEQ ID NO:1&2.

As described in Table 3, an animal with two copies of SEQ ID NO:1 does in fact have a significant increased milk production as compared to an animal with two copies of SEQ ID NO:2 over the whole lactation (TT v. CC – TT has 1.50 kg/day increased milk production over the CC –  $P=0.04$ ). An animal with one copy of SEQ ID NO:1 and one copy of SEQ ID NO:2 has a statistical trend of increased milk production when compared to an animal with two copies of SEQ ID NO:2 (CT v. CC – CT has increased milk production of 0.91 kg/day over the CC –  $P=0.12$  *statistical trend*). Also the reviewer claims that “there is not a statistically significant increase during the entire lactation, or during the time period of 200 days after the start of lactation (table 3).” Table 3 definitively describes TT (two copies of SEQ ID NO:1) animals in the 101-200 days in milk (DIM) period as having a statistically significant increase in milk production when compare to TC and CC animals ( $P=0.04$  for both TT v. CT [TT increased milk production of 1.74 kg/day]; and CT v. CC [CT increased milk production of 1.38 kg/day]).

SEQ ID NO's:1&2 contain the Arg25Cys polymorphism at position 189, which is the correct polymorphism site to detect the exon 2 C to T transition that is contemplated in this patent application. Accession No. AAE82807 is amino acid sequence as entered by Spurlock et al. SEQ ID NO's:1&2 are correct in describing the area which harbours the Arg25Cys polymorphism in the full length peptide, as described in SEQ ID NO:3.

The claims have been limited and now contain the limitation that the T containing allele results from a change from Arginine to Cysteine. Table 3 presents this data as analyzed to test for Main Model effects and to test whether or not “treatment” has a significant difference from the “control”. Since the C allele is the wild type, and the T allele is the mutant allele; then the C allele is the “control” and the T allele is the “treatment”. This analysis is set up whereby two different levels of treatment are analyzed v. the control – or CC (control) v. CT (1<sup>st</sup> level of treatment) v. TT (2<sup>nd</sup> level of treatment), and the P value is analyzing the Main Model effect. It is possible to test if there is a significant difference between CT (1<sup>st</sup> level of treatment) v. TT (2<sup>nd</sup> level of treatment) by, for example, use of a Tukey's test – but this has not been reported here. But biologically it is clear that TT animals do have a larger increase in milk production/day – and since the Main Model tests for “treatment” effect we can clearly know that the TT's have increased milk production when compared to the CT's.

Reference to the greater feed conversion efficiency has been deleted from the claims.

Also, as indicated, the claims have been restricted to bovine dairy cattle.

In the case of milk production, it is determined statistically (Table 3) that TT animals indeed have an increased state of milk production v. CC animals – and there is a statistical trend (declared at  $P<0.15$ ) for TT animals to have increased milk production over the CT animals ( $P=0.12$ ). While SEQ ID NO's:4-7 are not the sequence with the highest degree of homology with respect to SEQ ID NO's:1&2, they are nonetheless homologous to this region,

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and indeed span the region which encompasses position 189 of SEQ ID NO:1&2. These sequences (primers and probes) do not encompass all the polymorphisms in the *ob* gene, and it is not required to test all the polymorphism in the *ob* gene in order to use the contemplated claims, as in that case it is potentially likely that there would be a high proportion of linkage disequilibrium between the polymorphisms. Also it is inferred that the examiner is suggesting that repetition of experiments may somehow enhance the claims, when in fact while "replication" is a pillar of statistical design and analysis for efficacious experimental design and methodology – it is also incumbent on scientists to always conduct a "new" experiment to learn some new piece of knowledge rather than just repeating scientific results. MAY 29 2008

The examiner has also objected to the claims under 35 U.S.C. 102 as being anticipated by Konfortov *et al* and by GenBank. Reconsideration and withdrawal of the objection by the examiner is respectfully requested.

While Konfortov describes sequence variation in a table (pg 11144) whereby the genotypes are depicted as being segregated into different coloured squares, it is nowhere contemplated in this prior art about "a method of increasing milk production in a selected group of livestock animals of the same species comprising (a) determining a genetic predisposition of each animal to produce milk by determining their *ob* genotype" as in claim 8(a). In fact they do not in particular segregate the animals for any particular reason other than to display inherent sequence variation. They do not describe a method for segregating animals at all. Dairy animals are not mentioned anywhere, nor is anything to do with milk production or any dairy phenotypes related to production agriculture, and finally there is no mention or description of anything which may lead one to conclude or understand any relationship between *ob* genotype and milk production characteristics.

There is no association between the depiction of sequence variation as described by Konfortov and any milk production parameters, not the least of which is milk production in the first 100 days in milk. There is absolutely no description of utility of this sequence variation at all by Konfortov. Again, it is not inherently associated with milk production as suggested by the examiner. It is submitted that Konfortov does not contemplate the association anywhere in the reference nor does he contemplate any of the parts of segregating animals based on this novel information.

Konfortov only contemplates describing sequence variation, not a method of segregating animals in order to increase milk production (during any period of the lactation) based on each animals' individual exon 2 Arg25Cys polymorphism in the *ob* gene.

While it may teach the analysis of several breeds of cows, including Jersey, it makes no mention of Holstein, the No. 1 dairy breed worldwide. Also it does not teach any method of segregating these animals, independent of breed type, based on an animals' individual exon 2 Arg25Cys polymorphism in the *ob* gene in order to increase milk production.

The examiner describes the rejection based on "Konfortov *et al* comprising an analysis of a polymorphic variant of the *ob* (leptin) gene inherently associated with milk production". Konfortov does not contemplate milk production anywhere in his publication, nor an analysis based on segregation for increased live animal production traits; rather he describes a method of deducing allele sequence knowledge for description and understanding of sequence

variation in these two particular genes. There is never any mention of any association with any milk production characteristics

The applicant submits with respect that the amended claims are patentable and requests reconsideration and withdrawal of the objection by the examiner.

Favourable consideration is respectfully requested.

Respectfully submitted,

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